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HOX transcription factors and the prostate tumor microenvironment

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ABSTRACT

It is now well established that the tumor microenvironment plays an essential role in the survival, growth, invasion, and spread of cancer through the regulation of angiogenesis and localized immune responses. This review examines the role of the *HOX* genes, which encode a family of homeodomain-containing transcription factors, in the interaction between prostate tumors and their microenvironment. Previous studies have established that *HOX* genes have an important function in prostate cancer cell survival *in vitro* and *in vivo*, but there is also evidence that HOX proteins regulate the expression of genes in the cancer cell that influence the tumor microenvironment, and that cells in the microenvironment likewise express *HOX* genes that confer a tumor-supportive function. Here we provide an overview of these studies that, taken together, indicate that the *HOX* genes help mediate cross talk between prostate tumors and their microenvironment.

INTRODUCTION

In addition to cancer cells, tumor tissue contains a variety of host cells, extracellular matrix components, and secreted proteins that together constitute the tumor microenvironment[1]. Crosstalk between the tumor and its microenvironment has an important role in tumor development, including the recruitment of immune cells and vascular cells, both of which can have profound effects on the survival and spread of the tumor and are therefore targets for cancer therapy[2-4]. In this review, we consider the role of the HOX family of transcription factors in the interaction between prostate tumors and their microenvironment.

THE *HOX* GENES

Early embryonic development is characterized by a number of overlapping signaling events that give rise to stable transcriptional states and these in turn confer specific identities at both the cellular and tissue level. Many of the transcription factors that are responsible for regulating embryonic development were originally characterized by the distinct phenotypes caused by

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mutations in either their reading frame or regulatory regions, and one of the most notable examples of this are the *HOX* genes[5]. The *HOX* genes encode transcription factors that are characterized at the protein level by a highly conserved DNA-binding domain, known as the homeodomain, and their expression defines the identity of cells primarily along the anterior to posterior axis of the embryo, both in the main body and within organs and appendages[6]. Mammals have 39 *HOX* genes that are organized in 4 distinct chromosomal clusters named A, B, C, and D. The *HOX* genes are named on the basis of which cluster they are found in, and their position within the cluster. Thus for example HOXB1 is the 3' most member of the HOXB cluster, and its immediate 5' neighbor is consequently named HOXB2[7]. The clusters arose through multiple duplication events during the evolution of vertebrates, and consequently *HOX* genes at equivalent positions within each cluster (e.g. *HOXA1*, *HOXB1*, *HOXC1*, and *HOXD1*) share high levels of sequence identity beyond the conserved homeodomain region, and are referred to as paralogues[5]. The sharing of enhancer regions within clusters confers unusual regulatory properties on *HOX* genes, whereby the 3' members are expressed earlier in development (temporal collinearity) and more anteriorly (spatial collinearity) than their 5' neighbors[8].

HOX proteins can bind as monomers to DNA, although the affinity and specificity of binding are considerably increased through an interaction with other homeodomain-containing transcription factors including Pre-B-cell Leukemia Homeobox (PBX) and Myeloid Ecotropic Viral Integration Site 1 Homolog (MEIS) proteins[9]. Of these, PBX can bind to HOX proteins from paralogue groups 1-11[10-12], whilst MEIS binds to HOX9-13 proteins[13]. Despite this increased binding specificity, HOX proteins exhibit high levels of functional redundancy in some contexts due to extensive sequence identity between paralogue group members and 3' and 5' neighbors[14].

HOX gene expression generally reduces before birth and many adult cells show either low levels of expression, or no expression. Exceptions include cells that maintain proliferative capacity in the adult, for example stem cells, and most notably hematopoietic stem cells (HSCs), which are dependent on the continued expression of *HOXB4* for proliferation[15]. The subsequent differentiation of HSCs along different lineages and ultimately to mature blood cells is also dependent on distinct patterns of *HOX* gene expression[16]. Other adult processes that are known to be at least partly dependent on *HOX* genes include the menstrual cycle[17] and the differentiation of mesenchymal stem cells[18]. Over the last 20 years it has become increasing clear that *HOX* genes are also very highly dysregulated, and usually strongly over expressed in a wide range of haematological and solid malignancies compared to the cells form which these cancers originate[19,20]. The *HOX* genes have multiple functions in cancer, and can act both as tumor suppressors and oncogenes. Examples of the former include HOXA5, which can promote expression of the p53 tumor suppressor protein[21], and HOXC12, which promotes cellular differentiation in follicular lymphoma[22]. However, the majority of reports indicate that *HOX* genes have a pro-oncogenic role, including functions that support tumor growth and invasion such as angiogenesis, metastasis, and immune evasion[23]. At the cellular level, a generalized role for many HOX proteins in cancer appears to be to prevent apoptosis by inhibiting *cFos*[24-27] and dual specificity protease 1 (*DUSP1*) expression[26,28,29]. *DUSP1* is a tumour suppressor gene[30], and whilst *cFos* is generally considered to be a protoncogene, cFos protein can also induce apoptosis through the activation of the cell death ligand, FAS1[31-35]. Additional cellular functions of individual HOX proteins include DNA repair[36] and the regulation of the cell cycle[37]. It has also become apparent that the *HOX* genes function to modify the tumour microenvironment, and it is this aspect of their biology that we focus on here.

HOX GENES IN PROSTATE CANCER

The role of *HOX* genes in prostate cancer has in general been more extensively studied than for other solid malignancies. *HOXC4*, *HOXC5*, *HOXC6*, and *HOXC8* have all been found to be highly expressed in lymph node metastases[38], and *HOXC6* and *HOXC8* overexpression has also been demonstrated in primary tumors[25]. *HOXC8* expression was also shown to be higher in tumors with a higher Gleason score[39]. Of these 4 *HOX* genes, *HOXC6* is reported to be the most highly upregulated in primary, metastasized, and castrate-resistant prostate cancer, and the presence of *HOXC6* RNA in urine might be a diagnostic marker for prostate cancer and a potential monitoring tool for disease progression[40], and was shown to distinguish between high and low grade prostate tumors with a very high specificity when used in conjugation with a second urinary marker, *DLX1*[41]. In addition, disrupting the interaction between HOX proteins and their PBX cofactor using the competitive antagonist HXR9[23] causes apoptotic cell death in the prostate cancer-derived cell lines LnCaP, DU145, and PC3, and was shown to block the growth of PC3 tumors in a mouse xenograft model[25].

The most extensively studied *HOX* gene in prostate cancer is *HOXB13* due to its apparent role in androgen sensitivity. It has been shown to be highly expressed in androgen receptor (AR) positive prostate cancer-derived cell lines, but only at a very low level in AR negative cell lines[42,43], and to be strongly expressed in hormone-refractory tumors after initial treatment[44]. Furthermore, mutations in *HOXB13* are associated with an increased risk of prostate cancer. The G84E variant was found to significantly increase the risk of heredity prostate cancer[45], and was present in around 5% of families with at least one affected member[46]. A second variant, G135E was found to be associated with an increased risk of prostate cancer in Chinese men[47]. At the cellular level the functional significance of these variants remains unclear; for example, *HOXB13* G84E was not found to result in an appreciably different phenotype to the wild type gene when expressed in PNT2 cells[48]. However, a clear mechanistic basis for the pro-oncogenic role of *HOXB13* has arisen over the last few years [Figure 1]. HOXB13 protein can function both as a repressor and activator of transcription. It represses the p21WAF1/CIP1 (*p21*) tumor suppressor gene, which can block androgen-stimulated cell proliferation[49], and has also been shown to bind directly to the enhancer region of the *RFX6* gene, the product of which inhibits the proliferation, migration, and invasion of prostate cancer cells[50]. HOXB13 additionally represses prostate derived Ets factor (*PDEF*) expression, which in turn blocks the expression of matrix metalloproteinase 9 (MMP-9) and the anti-apoptotic protein survivin, and thus reduces the invasive potential of cells[51]. A further pro-oncogenic effect of HOXB13 is exerted through the upregulation of zinc transporters that in turn results in lower intracellular zinc concentrations. This reduces the level of inhibitor of NF-κB alpha (IκBα) and promotes NF-κBα signaling leading to increased invasion and metastasis[52]. Thus, HOXB13 exerts multiple tumor-promoting effects through the regulation of specific target genes.

In addition to their roles in regulating the proliferation and survival of prostate cancer cells, it has become apparent that the *HOX* genes are also instrumental in promoting changes to the tumor microenvironment that support metastasis and angiogenesis [Figure 2]. Each of these aspects will be considered in detail in

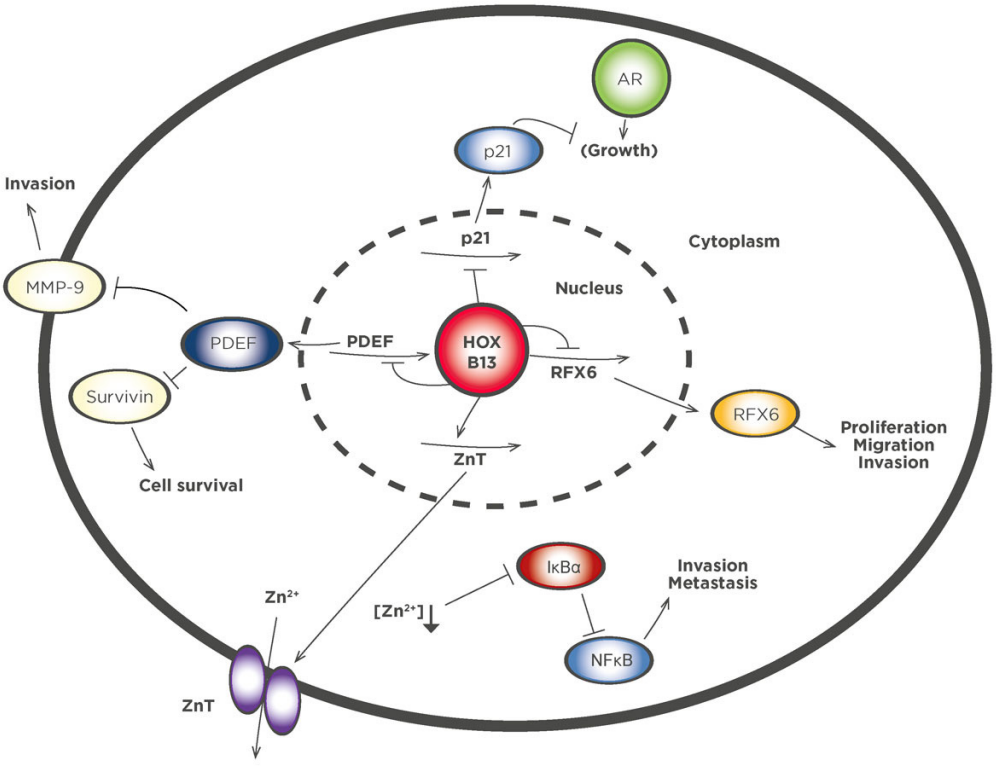

Figure 1: HOXB13 exerts multiple tumor-promoting effects through the regulation of specific target genes. HOXB13 protein can function both as a repressor and activator of transcription. It represses the p21WAF1/CIP1 (*p21*) tumor suppressor gene, which can block androgen-stimulated cell proliferation and has also been shown to bind directly to the enhancer region of the *RFX6* gene, the product of which inhibits the proliferation, migration, and invasion of prostate cancer cells. HOXB13 additionally represses prostate derived Ets factor (*PDEF*) expression, which in turn blocks the expression of matrix metalloproteinase 9 (MMP-9) and the anti-apoptotic protein survivin, and thus reduces the invasive potential of cells. A further pro-oncogenic effect of HOXB13 is exerted through the upregulation of zinc transporters resulting in lower intracellular zinc concentrations. This reduces the level of inhibitor of NF-κB alpha (IκBα) and promotes NF-κBα signaling leading to increased invasion and metastasis. Right pointing arrows in the nucleus indicate transcription. AR: androgen receptor

the remainder of this review.

HOX TRANSCRIPTION FACTORS AND METASTASIS

Metastasis is a complex, multi stage process and the tumor microenvironment plays a key role both at the earliest stages, in facilitating the movement of cells away from the primary tumor, and in the final stages in allowing metastatic cells to generate a new tumor at a distant site. One of the most important mechanisms by which tumors can modify the microenvironment is through the release of matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases that can modify the extra cellular matrix (ECM)[53]. Two of the most extensively studied of these enzymes with respect to prostate cancer are MMP-2 and MMP-9, both of which are members of the gelatinase subgroup of MMPs characterized by a fibronectin-like, gelatin-binding domain[54]. MMP-2 expression is higher in prostate tumors compared to normal prostate tissue, and has also been shown to be secreted by the former[55], and reducing its expression in mouse melanoma B16F10 cells resulted in significantly fewer lung metastases[56]. Both MMP-9 and MMP-2 expression is directly activated by the binding of HOXC11 protein to the enhancer region[57], and HOXC11 is expressed in multiple prostate cancer cell types[25] [Table 1]. MMP-9 expression has also been shown to be activated by HOXB7 in breast cancer cells[58], and both MMP-9 and HOXB7 are over expressed in prostate cancer[25,53]. The most frequently used prostate cancer-derived cell lines are LNCaP, DU145 and PC3, of which PC3 has by far the higher capacity for invasion *in vitro* and shows a significantly higher level of MMP-9 expression compared to the other cell lines[59]. Correspondingly, the invasive capacity of LNCaP increased significantly when MMP-9 was experimentally over-expressed in these cells[60], and invasion by DU145 and PC3 was reduced after MMP-9 expression was knocked-down using siRNA[61].

In addition to the gelatinase class MMPs, the expression of two other MMPs, MMP-3 and MMP-14, is activated by HOX transcription factors[62,63]. MMP-14 differs from other MMPs as it is membrane bound through a transmembrane domain with its catalytic center on the outside of the cell[64]. Its expression in prostate cancer cells is associated with androgen independence[65] and aggressiveness[66]. Prostate tumors primarily metastasize to bone, and MMP-14 has a particularly important role in this process due to

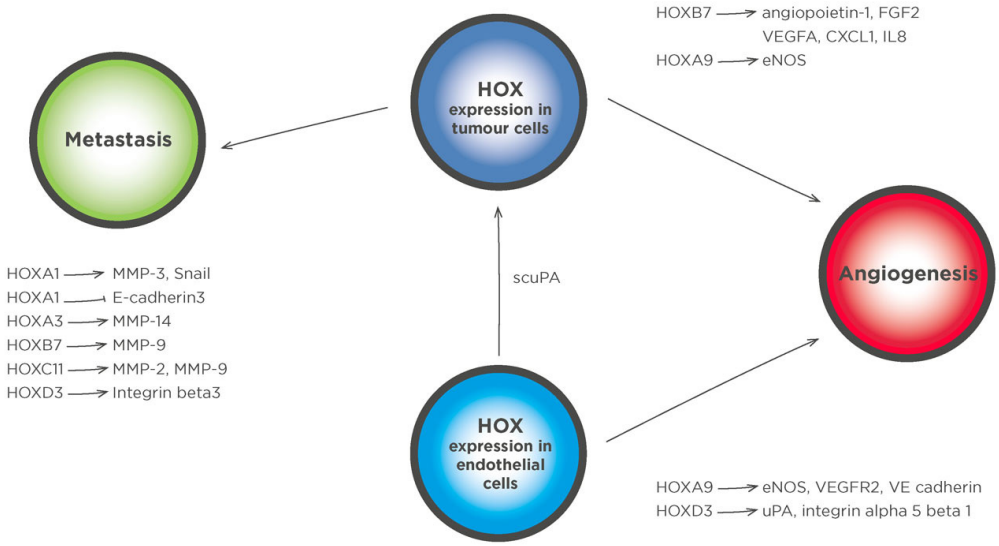

Figure 2: HOX transcription factors regulate genes in prostate cancer cells that modify the tumor microenvironment, as well genes in stromal cells that support tumor growth. HOX transcription factors have multiple roles in regulating genes that drive angiogenesis and metastasis. HOX targets with a key role in metastases include MMPs 2, 3, 9, and 14, as well as genes such as Snail and E-cadherin that are involved in the epithelial to mesenchymal transition. Genes involved in angiogenesis are also regulated by HOX transcription factors both in tumor cells and in endothelial cells. HOXD3 drives the expression of integrin alpha 5 beta 1 in endothelial cells which in turn leads to immature, leaky vessels. A number of HOX transcription factors can also drive the expression of proangiogenic secretory factors, including HOXB7, which regulates the transcription of FGF2, VEGFA, CXCL1, and IL8. An additional proangiogenic gene upregulated by HOXB7 is angiopoietin-1, the product of which plays a crucial role in stabilizing newly formed vasculature. Other proangiogenic genes that are regulated by HOX transcription factors include eNOs and uPA. HOXA9 expression in progenitor endothelial cells is necessary for their commitment to an endothelial lineage as it directly regulates endothelial specific genes such as eNOs, VE cadherin, and VEGFR2. HOXD3 has also been shown to have a role in vessel formation by endothelial cells through the activation of uPA transcription. In addition to an extracellular activity, a scuPA can be taken up by cancer cells in which it binds directly to HOXA5. MMP: matrix metalloproteinase; FGF2: fibroblast growth factor 2; VEGFA: vascular endothelial growth factor A; CXCL1: C-X-C motif ligand 1; IL8: interleukin 8; eNOs: endothelial nitric oxide synthase; uPA: urokinase plasminogen activator; scuPA: single chain form of uPA

Table 1: Direct and indirect regulation of target genes by *HOX* transcription factors in the context of the tumor microenvironment

HOX protein	Target gene	Direct or indirect regulation	Reference
HOXA1	*MMP-3*	Unknown	[63]
HOXA1	*Snail*	Unknown	[63]
HOXA1	*E-cadherin3*	Unknown	[63]
HOXA3	*MMP-14*	Unknown	[62]
HOXA9	*eNOS*	Direct	[99]
HOXA9	*VEGFR2*	Direct	[99]
HOXA9	*VE cadherin*	Direct	[99]
HOXB7	*MMP-9*	Unknown	[58]
HOXB7	*Angiopoietin-1*	Unknown	[58]
HOXB7	*FGF2*	Direct	[58,85]
HOXB7	*VEGFA*	Unknown	[58]
HOXB7	*CXCL1*	Unknown	[58]
HOXB7	*IL8*	Unknown	[58]
HOXC11	*MMP-2*	Direct	[57]
HOXC11	*MMP-8*	Direct	[57]
HOXD3	*Integrin beta 3*	Indirect	[75]
HOXD3	*uPA*	Unknown	[100]
HOXD3	*Integrin alpha 5 beta 1*	Direct	[82]

its ability to degrade collagen[67]. Accordingly, LNCaP cells overexpressing MMP-14 were shown to form significantly larger bone lesions in mice[67]. MMP-14 has been shown to be upregulated by *HOXA3* expression[62], and *HOXA3* is overexpressed in a number of cancers, including prostate cancer[25]. Another *HOX* gene linked to the progression of prostate cancer is *HOXA1*, the expression of which promotes the proliferation, invasion and metastasis of prostate cancer cells[63]. A number of key downstream target genes of HOXA1 have been identified, including *MMP-3*, which has itself been linked to prostate tumor progression in a number of studies[68-71], and polymorphisms in the *MMP-3* gene have been identified as a risk factor for the development of prostate cancer[72].

In addition to the *MMPs*, HOX transcription factors regulate a number of other target genes involved in the interaction of prostate cancers cells with the ECM. These include HOXA1, which inhibits the expression of E-cadherin[63], a major component of the epithelial adherence junctions that mediate intercellular interactions[73]. The downregulation of E-cadherin expression is one of the changes that occurs during the epithelial to mesenchymal transition, the activation of which in cancer cells is a key step in tumor invasion and metastasis[74]. The loss of E-cadherin also results in the disruption of the cytoplasmic cell adhesion complex, releasing proteins that can further modify the tumor microenvironment[73]. Another protein with a key function in cell adhesion is integrin β3, elevated expression of which is positively associated with high levels of *HOXD3* expression[75]. Integrin β3 has a role in tumor progression, invasion, and metastasis[76-78], and is also associated with more aggressive behavior of prostate cancer bone metastases[79]. Correspondingly, integrin antagonists have been shown to reduce bone degradation in clinical trials[80].

HOX TRANSCRIPTION FACTORS AND ANGIOGENESIS

Angiogenesis is a fundamental event in the natural history of tumors, allowing for their growth beyond a size restricted by the diffusion limits of nutrients and oxygen, and ultimately their systemic spread to form metastases[81]. HOX transcription factors have multiple roles in regulating the secretion of factors from tumor cells that drive this process in the microenvironment, and are also expressed in the cells of the tumor microvasculature in which they promote tumor-supportive functions. For the latter, HOXD3 has been shown to be particularly significant as it drives the expression of integrin alpha 5 beta 1 in endothelial cells which in turn leads to immature, leaky vessels that are typical of many tumor types[82]. Conversely, *HOXA5*, the expression of which results in more stable and less permeable vessels, is absent from tumor vessels[83,84]. Within tumor cells in has been shown that a number of HOX transcription factors can drive the expression of proangiogenic secretory factors. One of the earliest identified examples of this is *HOXB7*, which drives fibroblast growth factor 2 (FGF2, also known as bFGF) expression in multiple cancer types[58,85]. FGF2 is a well characterized proangiogenic factor, and has been shown to induce tubule formation by endothelial cells when secreted from a prostate tumor in a rat model of this disease[86]. In addition to FGF2, HOXB7 drives the expression of vascular endothelial growth factor A (VEGFA), C-X-C motif ligand 1 (CXCL1), and interleukin 8 (IL8)[58]. A role for IL8 in angiogenesis and its potential as a therapeutic target in cancer was demonstrated using fully-humanized antibodies to this protein in a mouse model of melanoma[87], and it was subsequently shown that IL8 increases expression of the key proangiogenic ligand VEGF in endothelial cells resulting in a self-reinforcing, autocrine loop through the VEGF receptor 2 (VEGFR2) expressed on the surface of these cells[88]. Correspondingly, polymorphisms in the *IL8* gene were shown to be associated with more aggressive prostate cancer[89]. CXCL1 is also a proangiogenic cytokine and has a potential role in the development of tumor resistance to anti-VEGF based therapy[90], and in gastric cancer has been shown to promote tumor growth through the VEGF pathway[91]. Correspondingly, the down regulation of CXCL1 has been shown to mediate the enhancement of the antiangiogenic effects of docetaxel by dexamethasone in *in vitro* and *in vivo* models of prostate cancer[92].

Its proangiogenic effects are also mediated through non-VEGF pathways, including the downregulation of fibulin-1 in castrate resistant prostate cancer[93]. It is targeted by the tumor-suppressor microRNA (miR)-200 that blocks angiogenesis and inhibits metastasis in multiple tumor types[94].

An additional proangiogenic gene upregulated by HOXB7 is angiopoietin-1 (*Ang-1*)[58], the product of which plays a crucial role in stabilizing newly formed vasculature. The binding of Ang-1 protein to its receptor on endothelial cells promotes their adherence to mural cells such as pericytes and smooth muscle cells[95-97]. Correspondingly, Ang1 secretion by prostate cancer cells in a xenograft model was shown to enhance tumor growth through an increased level of branching in the neovasculature[98].

Additional proangiogenic genes that are regulated by HOX transcription factors include endothelial nitric oxide synthase (eNOs)[99] and urokinase plasminogen activator (uPA)[100]. *HOXA9* expression in progenitor endothelial cells within the tumor microenvironment was shown to be necessary for their commitment to an endothelial lineage, and it was also shown to directly regulate endothelial specific genes such as *eNOs*, *VE cadherin*, and *VEGFR2*[99]. In this context *HOXA9* was identified as a key target of histone deacetylases (HDACs), as its expression was significantly reduced after HDAC inhibitor treatment and this in turn blocked angiogenesis both in mice[99] and in a clinical trial of combined HDAC and VEGF inhibitors for multiple cancers including advanced prostate cancer[101]. HOXD3 has also been shown to have a role in vessel formation by endothelial cells through the activation of *uPA* transcription[100]. uPA is involved at all stages of angiogenesis, including endothelial cell division, migration, the formation of stable vessels, and the regulation of vascular permeability through proteolytic degradation of the extracellular matrix[102-104]. This is mediated through intracellular signaling initiated by its binding to receptors including uPA receptor (uPAR; CD87), low-density lipoprotein receptor-related protein receptor (LRP/α2MR), and specific integrins[105-110]. In addition, uPA converts plasminogen into serine protease plasmin[111,112], which in turn breaks down matrix proteins and activates a number of MMPs[113-116]. uPAR-bound uPA has been shown in a number of studies to be localized to the leading edge of migrating cells[117-119] to help ensure a focused degradation of the ECM and liberate matrix-bound proangiogenic factors, including VEGF[120-122] and FGF2[123,124]. In addition to an extracellular activity, a single chain form of uPA can be taken up by cancer cells and be translocated to the nucleus[125] where it binds directly to HOXA5 protein and prevents it from activating the transcription of the key tumor suppressor gene *p53*[21]. Taken together, these studies imply the existence of a HOX-mediated feedback mechanism from the developing neovasculature to the tumor whereby HOXD3 promotes uPA expression in the endothelial cells, and this in turn blocks *p53* expression in the tumor, promoting cell proliferation and survival.

CONCLUSION

The evidence from previous studies indicates that the expression of *HOX* genes in the prostate tumor modifies the microenvironment in a manner that supports metastasis through degradation of the ECM, and angiogenesis through the secretion of proangiogenic cytokines. This is complemented by the expression of *HOX* genes in the microenvironment, particularly in endothelial cells, that promotes tumor-supportive functions including angiogenesis and the secretion of proteins that directly influence the malignant phenotype. Thus, targeting the function of HOX proteins may not only have a direct effect on tumor cells, but could also help reverse changes in the tumor microenvironment that would otherwise promote cancer progression.

DECLARATIONS

Authors' contributions

Performed the literature search and drafted the manuscript: R. Morgan

Helped write the manuscript and provided further interpretation of the referenced studies: H.S. Pandha

Financial support and sponsorship

None.

Conflicts of interest

The authors are shareholders in HOX Therapeutics Ltd., a company which is developing novel HOX/PBX binding antagonists, although these reagents are not discussed in this review.

Patient consent

Not applicable.

Ethics approval

Not applicable.

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